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13. ABSTRACT *(Maximum 200 Words)*

The genetic differences between aggressive metastatic and localized breast cancer are not known. However genetic instability is a poor prognostic factor in many types of cancer, implying that processes that lead to the gain, loss, or rearrangement of genomic DNA are important in the evolution of cancer. Telomeres are protein-DNA complexes that cap the ends of linear chromosomes, protecting them from degradation and fusion. In most cells a number of processes lead to the cumulative reduction of telomere length and cell cycle arrest. Some cells, including cancer progenitor cells, are able to up regulate telomerase, the enzyme that adds telomere repeats, and by pass cell cycle arrest.

The purpose of this study is to gain expertise in breast cancer research by determining if it is possible to differentiate patients with aggressive metastatic breast carcinoma from those with a less aggressive localized disease using telomere DNA content as a prognostic marker. To that end it is important to ascertain what effect telomerase has on telomere DNA content.

Here we report that high levels of the telomerase catalytic subunit, hTERT are correlated with several known prognostic markers including: tumor grade, S-phase, pliody and metastasis. Analysis of the relationship between telomere DNA content and hTERT expression is ongoing.

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Introduction:

The genetic differences between aggressive metastatic and less aggressive localized breast cancer are not known. However genetic instability is a poor prognostic factor in many types of cancer, implying that processes that lead to the gain, loss, or rearrangement of genomic DNA are important in the evolution of cancer. Telomeres are protein-DNA complexes that cap the ends of linear chromosomes, protecting them from degradation and fusion. In most cells a number of processes lead to the cumulative reduction of telomere length and cell cycle arrest. Some cells, including cancer progenitor cells, are able to up regulate telomerase, the enzyme that adds telomere repeats, and by pass cell cycle arrest. Interestingly, telomerase does not usually lengthen telomeres in cancer cells, instead it maintains them.

The purpose of this study is to gain training in breast cancer research by determining if it is possible to differentiate patients with aggressive metastatic breast carcinoma from those with a less aggressive localized disease using telomere DNA content as a prognostic marker. To that end it is important to ascertain what effect telomerase and its transcriptional activator c-myc have on telomere DNA content. Elucidating the role these proteins play in telomere length regulation is important if telomere DNA content is to be used as a prognostic tool and to further understanding of the mechanisms that drive carcinogenesis.

Tasks:

The agreed upon tasks to be completed in the statement of work were as follows:

1. Perform a preliminary retrospective investigation of the relationship between telomere DNA content and outcome in breast carcinoma.
2. Determine the relationship between telomere DNA content and c-myc amplification.
3. Determine the relationship between telomere DNA content and hTERT expression in breast carcinoma.

Progress Relative to the Statement of Work:

Task 1: A study population of 62 women diagnosed with breast cancer prior to 1995 was identified in cooperation with the New Mexico Tumor Registry (NMTR). The study population was selected such that approximately half of the women died of metastatic breast cancer, although these women had similar prognostic markers at the time of diagnosis as the control group who survived eight years or greater without signs of disease recurrence. Abstracted patient files were reviewed and available vital statistics were recorded including: age at diagnosis, tumor grade and size, estrogen/progesterone receptor status, treatment and length of survival or date and cause of death. Archival paraffin-embedded tumor and genetically matched normal tissue was obtained for a total of 50 women.

Tissue type and tumor grade were confirmed histologically by Dr Nancy Joste, Department of Pathology, University of New Mexico School of Medicine. DNA was extracted from a total of 100 paraffin-embedded blocks including both tumor and normal tissue from 50 women using the Quiagen Quiamp Tissue Kit (Quiagen). Purified DNA was quantified with a commercial fluorescence-based DNA detection reagent (Picogreen Molecular Bioprobes, Inc). It was not possible to purify DNA from four of the 50 women. To date, 10 tumor-normal pairs of tissue have been analyzed for telomere DNA content. Telomere DNA content analysis will be completed in the next two months.

Table 1: Comparison of Known Prognostic Markers for Study Group I

	Diagnosis	Age at Diagnosis	Tumor Grade	Positive Nodes	Positive Estrogen Receptor	Positive Progesterone Receptor	Years of Follow-Up
Good Outcome	Infil. Duct Carcinoma	**45** (58-35)	**2.4** (4-2)	21/24	9/14	11/14	**17** (21-8)
Poor Outcome	Infil. Duct Carcinoma	**49** (68-42)	**2.7** (4-2)	20/20	9/11	9/11	**14** (21-8)

Good Outcome patients are those who survived greater than eight year without disease recurrence. Poor Outcome patients are those women who died of metastatic breast cancer. Average is shown in bold, and the range for each category in parenthesis. The number of known patients with positive lymph node, estrogen or progesterone receptors is shown.

Task 2: The levels of c-myc in the tumor samples will be analyzed by quantitative RT-PCR. Since this proposal was submitted it has been possible to utilize a Real Time PCR technology. Since real time PCR is more sensitive and accurate than quantitative RT-PCR it will be employed to measure the levels of c-myc and hTERT. It was not possible to purify RNA, the starting component of real time PCR, from paraffin-embedded archival tissue; therefore a second study population was identified. Tissue from this second group was stored frozen and was therefore a good source of RNA. The second study population consisted of 70 women diagnosed with breast cancer prior to 1993. Tumor tissues were obtained from mastectomy, lumpectomy or biopsy. Because it was necessary to use only frozen tissue, the members in the second study population were not matched for age, specific type of breast tumor, tumor grade, age at diagnosis estrogen or progesterone receptor status or survival. Dr. Nancy Joste confirmed tumor grade and tissue type.

To date RNA and DNA have been purified from 60 of the 70 tumors. RNA was quantitated using absorbance spectra. DNA was quantitated as described previously. At this time, none of the samples have been analyzed for levels of c-myc. Probes for c-myc have been designed. C-myc levels will be measured in the next nine to eleven months.

Task 3: The second study group has been used as a source of RNA to measure the levels of hTERT expression in breast tumors. Thirty-six breast tumors have been analyzed for hTERT expression using real time PCR. There is a statistically significant correlation between high levels of hTERT expression and tumor grade =3, aneuploidy, positive lymph nodes and high % of cells in S-phase, as shown below.

Table 2: Correlates with hTERT Expression using Real Time PCR

Tumor Marker	P value
Tumor Grade	P=0.022
Ploidy	P=0.047
% S Phase	P=0.032
Positive Nodes	P=0.035

P values were calculated using a Pearson's Chi-Square Test

Telomere DNA content is being analyzed for the second study group and will be completed by July 1st.

Key Research Accomplishments:

- Identified a study population, half of whom survive greater eight years without disease recurrence and half of whom died from metastatic breast cancer. This study population had similar prognostic markers at the time of diagnosis.
- Purified and quantitated genomic DNA from tumor normal pairs of the first study population.
- Telomere DNA content has been analyzed in ten of tumor/normal tissues from the first study population.
- Identified a second study population, whose tissues have been frozen, thus preserving the integrity of tumor RNA.
- Developed real time PCR assay for hTERT, the catalytic subunit of telomerase, expression.
- Purified and quantitated genomic DNA and RNA from tumor tissues from the second study population.
- Completed analysis of 36 tumors for levels of hTERT expression using real time RT-PCR

Reportable Outcomes:

- Two databases have been produced that contain anonymous patient histories, including age at diagnosis, treatments, tumor grade, estrogen and progesterone receptor status, tumor size, length of disease free survival or date and cause of death and diagnosis.
- DNA and RNA banks from tumor and normal breast tissues have been produced.
- Tissue culture cell lines, which contain the hTERT and TBP genes that are used as controls for real time PCR have been produced.
- Ms Colleen Fordyce has been supported by this training grant. She has gained expertise in database development, DNA and RNA purification from archival tissues, slot and southern blots and real time RT-PCR methodologies.

Conclusions:

- hTERT levels correlate with several known prognostic markers including: tumor grade, ploidy, % of cells in S-phase, and metastasis effectively identifying the subset of tumors with the worst prognostic markers. Therefore hTERT may be a "meta-prognostic marker" and could be used to identify the most aggressive tumors.